Matrix-assisted laser desorption/ionization time of flight, MALDI-TOF, mass spectrometry for identification of fish pathogenic bacteria

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Matrix-assisted laser desorption/ionization time of flight, MALDI-TOF, mass spectrometry for identification of fish pathogenic bacteria

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Introduction. MALDI-TOF has recently been implemented as a new, fast and cost effective tool for identification of bacteria in diagnostic laboratories. Bacterial colonies grown on agar plates are tested, and with the MALDI-TOF technique, an unique spectrum is obtained, which is then compared with reference spectra in a database. The quality of the identification is dependent on the extent and quality of the database. In the present collaborative study, fish disease laboratories in Denmark (DTU Vet), Netherlands (CVI) and Sweden (SVA) developed Main Spectra Projections (MSPs) for important fish pathogens from the genera *Aeromonas*, *Flavobacterium*, *Yersinia* and *Vibrio* for their own collaborative database to improve the possibility to identify important fish pathogens.

Materials and methods. Main spectra projections, (MSPs) were produced at CVI, DTU-Vet and SVA for *Aeromonas salmonicida* (11 MSPs), *Flavobacterium columnare* (8 MSPs), *Flavobacterium psychrophilum* (16 MSPs), *Yersinia ruckeri* (3 MSPs), *Vibrio anguillarum* (8 MSPs) and one of each of *Vibrio ichthyoenteri*, *Vibrio splendidus* and *Vibrio vulnificus* according to recommendations by the manufacturer, Bruker Daltonics. Isolated bacteria from routine diagnostics and from the laboratories own collections, previously identified by standard techniques as biochemical assays and/or 16S rRNA sequencing/PCR, were cultivated on blood agar (*A. salmonicida, Y. ruckeri, V. anguillarum,* and *V. vulnificus*) or on TYES agar (*F. columnare* and *F. psychrophilum*). Identification by MALDI-TOF was performed according to the manufacturer´s instructions. In short, a fresh bacterial colony was picked by a toothpick and transferred to a spot on the MALDI target plate. Matrix solution was added, let to dry and the MALDI target plate was inserted into the MALDI-TOF spectrophotometer for exposure to the laser beam. The obtained digital spectra (Fig.1.) were compared with spectra in Bruker´s database and own produced MSPs. A high degree of similarity with the known bacterial spectra in the databases gives a "score" in the program. A "score" above 2.0 indicate a secure identification to the genus level and probably also to the species level. A "score" above 2.3 indicate a secure identification to the species level, Fig. 2.

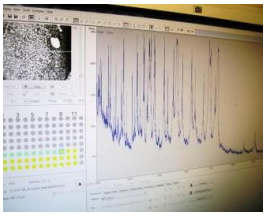

Fig. 1. Screen shot: Digital spectra obtained from the bacterium under test.

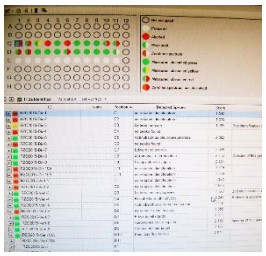

Fig 2. Screen shot: Score based pattern matching, most similar bacteria is given.

Results and discussion
Colony morphology in combination with MALDI-TOF was found to be a safe and useful approach for identification of the fish pathogenic bacteria *F. columnare*, *F. psychrophilum*, *V. anguillarum*, *V. vulnificus*, and *Y. ruckeri*. On the contrary**,** identification of bacteria of different subspecies of *A. salmonicida* were not correctly identified in the present database, not even after complementation with new MSPs. This will be the aim of future studies.

Table 1. Identification of fish pathogenic bacteria by MALDI-TOF by use of Bruker´s database complemented with own produced Main Spectra Projections (MSP's)

Bacteria, identified by standard techniques as biochemical assays or PCR	Number of tested isolates	Identification by MALDI-TOF	Score
Aeromonas salmonicida[1]	13	*Aeromonas salmonicida*, 8 isolates *Aeromonas bestiarum*,4 isolates *Aeromonas eucrenophila,* 1 isolates	2.13 – 2.302 1.917 – 2.214 2.174
Flavobacterium columnare[2]	17	*Flavobacterium columnare*	2.135 – 2.417
Flavobacterium psychrophilum[2]	59	*Flavobacterium psychrophilum*	2.204 – 2.544
Vibrio anguillarum[3]	8	*Vibrio anguillarum*	2.123 – 2.318
Vibrio vulnificus[1]	30	*Vibrio vulnificus*	2.029-2.468
Yersinia ruckeri[3]	11	*Yersinia ruckeri*	1.997 – 2.239

[1]MSPs already in Bruker´s database complemented with own produced MSPs in the present study.
[2] MSPs produced in the present study as no MSPs for the actual species are available in Bruker´s database.
[3]MSPs already in Bruker´s database.